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1. Your reference — PJS/P9845GB

2. Patent application number *(The Patent Office will fill this part in)* — 0330009.2 — 24 DEC 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

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Denmark

Patents ADP number *(if you know it)* 8779191001

If the applicant is a corporate body, give the country/state of its incorporation — Denmark

4. Title of the invention — PROBIOTIC TABLET FORMULATIONS

5. Name of your agent *(if you have one)* — W. H. Beck, Greener & Co.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Patents ADP number *(if you know it)* 323001

6. Priority: Complete this section if you are declaring priority from one or more earlier patent applications, filed in the last 12 months.

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. Divisionals, etc: Complete this section only if this application is a divisional application or resulted from an entitlement dispute (see note f)

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8. Is a Patents Form 7/77 (Statement of inventorship and of right to grant of a patent) required in support of this request?

Answer YES if:

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
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Otherwise answer NO (See note d)

Yes

9. Accompanying documents: A patent application must include a description of the invention. Not counting duplicates, please enter the number of pages of each item accompanying this form:

Continuation sheets of this form	
Description	16
Claim(s)	6
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10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent (Patents Form 7/77)	
Request for a preliminary examination and search (Patents Form 9/77)	
Request for a substantive examination (Patents Form 10/77)	
Any other documents (please specify)	

11. I/We request the grant of a patent on the basis of this application.

Signature(s) Date 24.12.03

12. Name, daytime telephone number and e-mail address, if any, of person to contact in the United Kingdom

Mr Peter J. Smart - (020) 7693 5600

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Probiotic tablet formulations

The present invention relates to the formulation of probiotic micro-organisms in tablet form. Probiotic micro-organisms are conventionally formulated with other nutritionally active materials such as vitamins, minerals, carbohydrates, proteins, co-enzymes, enzymes, plant extracts, trace elements, and/or fats. Whilst many probiotic micro-organisms are quite stable when kept by themselves in a dried form, tablet formulations in which the probiotic micro-organisms are mixed with active ingredients of the above kinds are highly unstable. After even brief storage, the recovery of viable micro-organisms upon rehydration of such mixed formulations will be extremely poor.

US6254886 attempts to address this problem by proposing that the tablet should be in a multilayer form with the probiotic micro-organism being contained in a layer which is free from other nutritionally active materials and which is dry to the extent that its water content is less than 0.1%. Since water is in fact free to move between the different layers of the tablet, this in practice means that the carrier material for all the tablet layers has to be dry to this same extent. Moreover, where large amounts of other active ingredients are present, they too will have to be aggressively dried if the total water content of the probiotic layer is not to rise significantly above the limits set in US6254886.

We have now found that the water content in a storage stable probiotic tablet formulation can be very much higher than is taught in US6254886 provided that care is taken that the water activity is maintained below 0.2 (equivalent to 20% relative humidity) and that the mixing with the probiotic micro-organisms of certain active materials taught to be kept separate from the probiotic micro-organisms in US6254886, is not deleterious and may actually improve the viability of the micro-organisms.

The present invention now provides a probiotic tablet comprising a probiotic micro-organism and other nutritionally active ingredients, the tablet comprising at least two zones, a first of said zones comprising said probiotic micro-organism, and a second of said zones comprising at least one said other active ingredient kept separated from the probiotic micro-organism of said first zone, the water activity in said probiotic micro-organism containing first zone being no greater than 0.2 and the water content of said tablet being no less than 0.2% by weight.

Tablets according to the invention, particularly as exemplified below may be storage stable at a cool temperature (up to 15 °C) or more preferably at room temperature (up to 20 °C or more preferably up to 25 °C) for several months, e.g. for up to one year or more preferably up to 18 months or more preferably two years or more. By 'storage stable' is meant that after a storage period, the number of viable probiotic micro-organisms should not have declined by more than a factor of one thousand, preferably not more than one hundred, more preferably not by a factor of more than 10 e.g.. from $5*10^9$ to $5*10^8$, or less preferably to $5*10^7$ or still less preferably to $5*10^6$.

According to US 6254886, the presence together with the probiotic micro-organism of other substances valuable in nutritional physiology is deleterious. It is suggested that at best there may be some unidentified active materials that are not deleterious. However, we have found that certain active materials actually improve the stability of the product when they are present in the first zone. In accordance with this, it is preferred that said first zone contains also selenium as a said at least one other active ingredient . Preferably, said first zone contains from 1 to 100 µg , e.g. 5 to 75 µg, more preferably 7.5 to 60 µg, of selenium, per 10^9 micro-organisms.

The presence of selenium together with the micro-organisms is particularly preferred as we have demonstrated that selenium increases the storage stability of the tabletted micro-organisms. The mechanism responsible for this is at present uncertain. It may be that the selenium exerts a beneficial influence in one or more of several ways including as a growth medium, as a compression distributor, as a stabiliser, as a desiccant or as an antioxidant.

The presence in said first zone of antioxidants generally is also preferred. These include ascorbyl palmitate or other ascorbates, propyl galates or other –gallates, alpha-tocopherol, magnesium or sodium sulfite, butylated hydroxyanisole or butylated hydroxytoluene.

Certain active ingredients are however deleterious and should preferably be excluded from the first zone. These include iron, vitamin B6, vitamin C, zinc, copper, manganese, chromium, pantothenic acid or its salts, and to a lesser extent vitamin B1, so the first zone is preferably free from amounts of some or all of each of these that are sufficient materially to exert an adverse effect on the stability of the product. Several of these materials are available in a micro-encapsulated form. One way in which such materials may be present in a tablet according to the invention without

their being present in the first zone is for them to be encapsulated, but to be present as micro-particles mixed in to the probiotic micro-organism containing material. If the level of separation imposed by the micro-encapsulation of these materials is not adequate, they may still exert an adverse effect, so we prefer that they should not be mixed into the first zone in micro-encapsulated form, but should be relegated to a more physically distinct and separate macro-region of the tablet, such as a distinct layer. This applies especially to iron and copper.

Encapsulated zinc is better tolerated and can be admixed into the first zone materials.

Vitamin B1 can be present in the first zone in non-encapsulated form without much deleterious effect.

Some benefit may come from having certain encapsulated materials mixed into the first zone. These include micro-encapsulated vitamin B1, micro-encapsulated vitamin B6, micro-encapsulated zinc, micro-encapsulated manganese, micro-encapsulated vitamins A, D, E, B12 and B2.

Said second zone preferably contains as at least one said other active ingredient any one of iron, vitamin B6, vitamin C, zinc, copper, manganese, chromium, and pantothenic acid or a salt thereof. Preferably at least two, more preferably at least four, more preferably at least six and preferably all of these are present.

It is preferred that the tablets of the invention have a multi-layer form comprising two or more layers, one of said layers constituting said first zone and another of said layers constituting said second zone. Additional layers may be present. The layers may be formed one over the other or such that a body of material constituting one of the first and second zones is enrobed by a layer of material constituting the other of said zones.

Where such a two layer structure is used, it is still possible for the layer constituting said first zone to contain in encapsulated form some materials which are required to be kept out of the first zone, but for better separation of the probiotic micro-organisms from these materials it is preferred that they are not present mixed within the first zone layer but are present only in the second zone. This reduces the interface area between zones containing the probiotic micro-organism and these potentially destabilising ingredients. These include particularly iron, encapsulated iron, vitamin B6, vitamin C, zinc, copper, manganese, chromium, pantothenic acid

and its salts, and encapsulated copper and to a lesser degree encapsulated zinc, especially if not strongly encapsulated, and vitamin B1.

On the other hand, it may be acceptable or even beneficial if mixed within the layer constituting the first zone are one, two or any combination of micro-encapsulated vitamin B1, micro-encapsulated vitamin B6, selenium, micro-encapsulated zinc, iodine, micro-encapsulated vitamins A, D, E, B12 or B2, nicotinamide, folic acid, or any of the anti-oxidants mentioned herein.

Summing this up, if one were to categorise other active ingredients likely to be present into three lists: A(aggressive ingredients to be kept well away from the probiotic material, e.g. in a separate layer), B (somewhat aggressive ingredients which are preferably excluded from the first zone, but which may well be tolerated either in the first zone or in micro-encapsulated form surrounded by the first zone) and C (non-aggressive or beneficial ingredients that can be present in the first zone or if encapsulated can be surrounded by the first zone) these lists would be as follows:

<u>List A</u>

iron

Encapsulated Fe

Vitamin B6

Vitamin C

Zinc

Copper

Manganese

Chromium

Calcium pantothenate

Encapsulated copper

<u>List B</u>

Encapsulated zinc

Vitamin B1

<u>List C</u>

Encapsulated vitamin B1

Encapsulated vitamin B6

Selenium

Encapsulated zinc

Iodine

Magnesium

Encapsulated manganese

Encapsulated vitamin A, D, E, B12, B2

Nicotinamide

Folic acid

Whilst layer structures are preferred, it is permissible for the tablet to have a multitude of granules constituting said first zone surrounded by a matrix, wherein said matrix constitutes said second zone or wherein said matrix also contains a multitude of granules constituting said second zone.

In order to obtain a low water activity in the first zone, the probiotic micro-organism is preferably mixed with a desiccant carrier material serving to reduce the water activity of the zone containing the probiotic micro-organism. Optionally however such a desiccant carrier material serving to reduce the water activity of the zone containing the probiotic micro-organism may be present instead in the second zone. Preferably, such a material is present in both the first and the second zones. The effect of such a desiccant may be to sequester part of the water content of the zone so that it is no longer in the form of free water that can migrate into the probiotic micro-organisms and is therefore prevented from carrying active substances through the cell walls of such organisms. Such desiccants bind water to specific sites so that it is no longer able to act as a solvent. These sites include the hydroxyl groups of polysaccharides, the carbonyl and amino groups of proteins, and others on which water can be held by hydrogen bonding, by ion-dipole bonds, or by other strong interactions. Thus, preferred desiccants include at least one of carboxymethylcellulose, colloidal silica, polyvinylpyrrolidone, starch, gelatine, hydroxypropylcellulose, microcrystalline cellulose, fumed silicon dioxide, sodium croscarmellose, crospovidone, povidone, magnesium aluminium silicate, methylcellulose, sodium alginate, sodium starch glyconate, gelatine, pregelatinized starch, or sorbitol. The desiccant may be in particular, a starch selected from corn, rice, or potato starch, a hydrophilic gum, polysaccharide or galactomannan such as pectin, agar, dextran, maltodextrin, carageenan, tragacanth gum, locust bean gum, acacia gum, guar gum, xanthan gum, ghatti gum, alginic acid or sodium alginate, a

cellulose derivative such as methyl cellulose, carboxymethylcellulose, sodium starch glycollate, sodium or calcium carboxymethylcellulose, hydroxyethyl methylcellulose, hydroxypropylmethylcellulose, ethylhydroxyethylcellulose, ethylmethylcellulose, hydroxyethylcellulose, cellulose acetate phthalate, or microcrystalline cellulose, silica, aluminium silicate, magnesium silicate, aluminium magnesium silicate, sodium silicate or feldspar, aluminium hydroxide, a protein such as gelatin or casein or a polymer such as acrylate, carboxypolymethylene, a polyalkylene glycol or polyvinylpyrrolidone. Other steps to reduce the amount of oxygen present may be beneficial, including packing under an inert atmosphere such as nitrogen and the use of oxygen barrier packaging materials such as aluminium tubes or high barrier polymers.

The water content of the tablet is at least 0.2% by weight and may be considerably higher. Higher water contents remove the need for aggressive drying of materials which may be sensitive to such a process. It is undesirable that the water content in the tablet is too high as it increases the risk of unforeseen re-crystallisation. Also, it is expensive to remove water. Thus, the water content can be above 0.5% or above 1%, but below 6% more preferably below 5%, or 4%, 3% ,or even 2%. Alternatively, the water content can be above 0.5% or above 1% or 2% , but below 6% more preferably below 5%, or 4%, or 3%. Alternatively, the water content can be above 0.5% or above 1% or 2% or 3%, but below 6% more preferably below 5%, or 4%. The water content can go up to 7% by weight.

At the same time, the water activity is preferably below 0.18, more preferably below 0.15, still more preferably below 0.13, e.g. 0.10, or even 0.08. The water activity may be still lower, e.g. 0.05 or even 0.02. The water activity may lie between 0.2 and any of the foregoing figures or between any two of them.

Each of the foregoing figures for water activity relate to the first zone of the tablet. Normally, following internal equilibration, this will also be the water activity of the tablet as a whole. Unless an internal water excluding barrier layer is present separating off the first zone, the water activity will equilibrate throughout the tablet to reach the same value throughout.

To improve the separation of the probiotic micro-organisms from the ingredients that are hostile to their stability, said first zone may be separated from said second zone by a water excluding barrier material. Additionally or instead, the tablet as a whole may be surrounded by a water excluding material. Such materials may be

water soluble materials such as cellulose acetate phthalate, methacrylic acid copolymers or shellac.

The barrier materials may more preferably be or include a fat based material, which may be applied by a process of hot melt coating. These include but are not limited to fatty acid triglycerides, e.g. hydrogenated palm oil or beef tallow and mixtures of triglyceride esters of higher saturated fatty acids along with varying proportions of mono- and di- glycerides, e.g. hard fats.

Tablets according to the invention may be stored in a container containing a desiccant for absorbing water so as to reduce the water activity in the area surrounding said tablet. Thus, the tablets may be packaged in such a way as to preserve their initial state of dryness within acceptable limits. This may involve packaging the tablets in a moisture impermeable container such as a tube or a blister pack, which may contain a desiccant agent such as silica gel . For protection against oxygen such a pack may contain an oxygen scavenger material such as Amosorb™ , ascorbyl palmitate or other ascorbates, propyl galates or other –gallates, alpha-tocopherol, magnesium or sodium sulfite, butylated hydroxyanisole or butylated hydroxytoluene. Oxygen absorbents as described in US-A-5885481, 5744056, or 6083585 can be used.

The tablets may contain additional materials, especially in the second zone, such as plant materials, including herb materials, for example Echinacea, elderberry extract, blueberry extract, cranberry extract and rose hip.

The term 'probiotic micro-organism' is well understood by those skilled in the art to which this invention pertains. Probiotics are live micro organisms, normally freeze dried, which have a beneficial effect on health when ingested. The probiotic micro-organisms may be lactic acid producing bacteria, e.g. . *Lactobacilli* and *Bifidobacteria* bacteria. Probiotic micro-organisms that may be present include but are not limited to *Lactobacillus casei, Lactobacillus acidophilus, Bifidobacterium bifidum, Bifidobacterium longum, Bacillus coagulans, Saccharomyces boulardii, Saccharomyces cerevisiae, Lactobacillus paracacei, Lactobacillus plantarum, Lactobacillus rhamnosus, Lactobacillus reuteri, Lactobacillus gasseri, Lactobacillus jensenii, Lactobacillus delbruekii, Lactobacillus helveticus, Lactobacillus bulgaricus, Lactobacillus salivarius, Lactobacillus delbrueckii, Bifidobacterium adolescentis, Bifidobacterium animalis, Bifidobacterium infantis, Streptococcus thermophilus* and *Enterocossus faecium.*

Each tablet suitably will contain from 10^7 to 10^{12}, e.g. from 10^8 to 10^{10}, viable micro-organism cells.

Preferred methods for producing tablets from the tablet ingredients include standard tabletting methods, including those conventionally used for producing multi-layer tablets.

The tablets may be designed to be chewed or to be swallowed whole.

The invention will be further described with reference to the following illustrative examples of multilayer tablets, containing freeze dried probiotic cultures and vitamins/minerals, herbals or drugs.

Example 1

The following ingredients were formulated into a two layer tasty chewable tablet incorporating lactic acid bacteria, vitamins and minerals using Xylitol and Isomalt to provide bulk and sweetening:

Per tablet:

Vitamin A	mcg	700.00	Retinolacetate
Vitamin D	mcg	5.00	Cholecalciferol
Vitamin E	IU	10.43	D,L-alfa-tocopherolacetate
Vitamin B1(salt)	mg	1.00	Thiaminenitrate
Vitamin B2	mg	1.20	Riboflavin
Vitamin B6(salt)	mg	1.10	Pyridoxine chloride
Vitamin B12	mcg	1.40	Cyanocobalamin
Nicotinamide	mg	13.00	Nicotinamide
Pantothenic acid	mg	5.00	D-Calcium pantothenate
Folic acid	mcg	100.00	Folic acid
Vitamin C	mg	60.00	Ascorbic acid
Calcium	mg	200.00	Calcium carbonate
Magnesium	mg	50.00	Magnesium oxide
Iron	mg	10.00	Ferrous fumarate
Zinc	mg	7.00	Zinc oxide
Copper	mg	0.70	Cupric oxide

Manganese	mg	2.00	Manganese sulfate
Chromium	mcg	50.00	Chromium (III) chloride
Selenium	mcg	30.00	Sodium selenate
Iodine	mcg	90.00	Potassium iodide
Biotin	mcg	30.00	d-Biotin
Vitamin K	mcg	30.00	Phytomenadione
Lactobacillus GG cfu		$1x10^9$	

The vitamins and minerals (except for selenium) are mixed with the following excipients:

Xylitol	320 mg
Microcrystalline cellulose	64 mg
Flavour	33 mg
Stearic acid	22 mg
Silicon dioxide	7 mg
Acesulfam potassium	2 mg
(in total	700 mg)

The freeze dried probiotic culture (10 mg = $3x10^9$) and the selenium is mixed with:

Isomalt	253 mg
Xylitol	100 mg
Microcrystalline cellulose	31 mg
Magnesium stearate	4 mg
silicon dioxide	2 mg
(in total	400 mg)

Tablets were produced having two superposed layers using a conventional tabletting machine, the ingredients of one layer being filled over the ingredients of the other.

Tablet weight	1100 mg
Tablet size	11 by 16.5 mm oval
Water activity** in culture granulate	<0.1
Water content* in culture granulate	....
Water activity** in tablet	< 0.15

Water content* in tablet ....

** Nova Sina..., * Karl Fisher

Example 2

The following ingredients were formulated as a two layer tablet to swallow with lactic acid bacteria, vitamins and minerals.

Per tablet:

Vitamin D	mcg	5.00	Cholecalciferol
Vitamin E	IU	14.90	D,L-alfatocopherolacetate
Vitamin B1(salt)	mg	5.00	Thiaminenitrate
Vitamin B2	mg	5.00	Riboflavin
Vitamin B6(salt)	mg	5.00	Pyridoxinchloride
Vitamin B12	mcg	3.00	Cyanocobalamin
Biotin	mcg	30.00	d-Biotin
Nicotinamide	mg	18.00	Nicotinamide
Pantotensyre	mg	5.00	D-Calciumpantothenate
Folic acid	mcg	400.00	Folic acid
Vitamin C	mg	90.00	Ascorbic acid
Magnesium	mg	90.00	Magnesium oxide
Zink	mg	15.00	Zinkoxid
Manganese	mg	2.50	Manganese sulfate
Chromium	mcg	30.00	Chromium (III) chloride
Selenium	mcg	50.00	Sodium selenate
Iodine	mcg	100.00	Calcium iodide
Lactobacillus GG	cfu	$1x10^9$	

The vitamins and minerals (except for selenium) are mixed with the following excipients:

Microcrystalline cellulose	58 mg
Magnesium stearate	4 mg
Stearic acid	3 mg

Silicon dioxide	1 mg
(in total	555 mg)

The freeze dried probiotic culture (10 mg = $3x10^9$) and the selenium are mixed with:

Microcrystalline cellulose	183 mg
Magnesium stearate	2 mg
Silicon dioxide	0.4 mg
(in total	195 mg)

Tabletting was conducted as in Example 1 and the 2-layer tablets were filled into aluminium tubes with desiccant in the lid.

Tablet weight	750 mg
Tablet size	12 by 4 mm circular
Water activity** in culture granulate	0.07
Water content* in culture granulate	2%
Water activity** in tablet	0.07
Water content* in tablet	3.2%

** Nova Sina..., * Karl Fisher

Example 3

The following ingredients were formulated into a two layer tasty chewable tablet incorporating lactic acid bacteria, vitamins and minerals using Xylitol and Isomalt to provide bulk and sweetening:

Per tablet:

Vitamin A	mcg	700.00	Retinolacetate
Vitamin D	mcg	5.00	Cholecalciferol
Vitamin E	IU	10.43	D,L-alfa-tocopherol acetate
Vitamin B1(salt)	mg	1.00	Thiaminenitrate
Vitamin B2	mg	1.20	Riboflavin
Vitamin B6(salt)	mg	1.10	Pyridoxine chloride

Vitamin B12	mcg	1.40	Cyanocobalamin
Nicotinamide	mg	13.00	Nicotinamide
Pantothenic acid	mg	5.00	D-Calcium pantothenate
Folic acid	mcg	100.00	Folic acid
Vitamin C	mg	60.00	Ascorbic acid
Calcium	mg	200.00	Calcium carbonate
Magnesium	mg	50.00	Magnesium oxide
Iron	mg	10.00	Ferrous fumarate
Zinc	mg	7.00	Zinc oxide
Copper	mg	0.70	Cupric oxide
Manganese	mg	2.00	Manganese sulfate
Chromium	mcg	50.00	Chromium (III) chloride
Selenium	mcg	30.00	Sodium selenate
Iodine	mcg	90.00	Potassium iodide
Biotin	mcg	30.00	d-Biotin
Vitamin K	mcg	30.00	Phytomenadione
Lactobacillus GG	cfu	$1x10^9$	

The vitamins and minerals (except for selenium) are mixed with the following excipients:

Lactitol	209 mg
Microcrystalline cellulose	39 mg
Flavour	2.5 mg
Stearic acid	44 mg
Silicon dioxide	14 mg
Neohesperidin 10%	0.2 mg
Citric acid monohydrate	2 mg
(in total	1160 mg)

The freeze dried probiotic culture (10 mg = $3x10^9$) and the selenium is mixed with:

Lactitol	394 mg
Microcrystalline cellulose	21 mg
Stearic acid	14 mg
(in total	440 mg)

Tabletting was conducted as in Example 1 and the 2-layer tablets were filled into aluminium tubes with desiccant in the lid.

Tablet weight	1600 mg
Tablet size	16 mm circular
Water activity** in culture granulate	<0.1
Water content* in culture granulate	....
Water activity** in tablet	0.09
Water content* in tablet	3.7%

** Nova Sina..., * Karl Fisher

In the above Examples, the vitamins used were in some cases supplied in an encapsulated form, others were used in non-encapsulated form. The table below indicates the ingredients present in the vitamin formulations used

Active ingredients	**Amount**
Vitamin D (Cholecalciferol)	**5 mcg = 200 IU**
As Cholecalciferol Concentrate Powder (analysed to 110 IU/mg)	2.00 mg
- Cholecalciferol	6 mcg
- Sucrose	0.68 mg
- Gelatin	0.42 mg
- Modified Starch	0.42 mg
- Triglycerides, medium-chain	0.38 mg
- Butyl Hydroxytoluene	19 mcg
- Sodium Aluminosilicate	3 mcg
- Water	72 mcg
Vitamin E (D-α-tocopherol)	**14.90 IU**
As α-Tocopherol Acetate Concentrate (Powder form)(analysed to 52,5 w/w %)	30.08 mg
- DL-α-Tocopherol Acetate	15.79 mg

Active ingredients	**Amount**
- Maize Starch	6.02 mg
- Gelatin	5.11 mg
- Sucrose	1.41 mg
- Sodium Aluminosilicate	0.39 mg
- Water	1.35 mg
Vitamin B1 (Thiamin)	**5 mg**
As Thiamin Nitrate 33%	14.85 mg
- Thiamin nitrate	4.95 mg
- Mixture of mono-, di and triglycerides	9.90 mg
Vitamin B2 (Riboflavin)	**5 mg**
As Riboflavine 33%	15.60 mg
- Riboflavine	5.20 mg
- Mixture of mono-, di and triglycerides	8.84 mg
- Maize Starch	1.56 mg
Vitamin B6 (Pyridoxine)	**5 mg**
As Pyridoxine Hydrochloride 33%	15.45 mg
- Pyridoxine Hydrochloride	5.15 mg
- Mixture of mono-, di and triglycerides	10.30 mg
Vitamin B12	**3 mcg**
As Cyanocobalamine 0.1% (analysed to 0.11%)	1.87 mg
- Cyanocobalamine	3 mcg
- Maltodextrin	2.64 mg
- Sodium citrate	27 mcg
- Citric acid	20 mcg
- Water	120 mcg
Biotin	**30 mcg**
As D-Biotin	32 mcg
Nicotinamide	**18 mg**

Active ingredients	**Amount**
As Nicotinamide 33%	56.16 mg
- Nicotinamide	18.72 mg
- Mixture of mono-, di and triglycerides - Silicon dioxide	31.82 mg 5.62 mg
Pantothenic Acid	**5 mg**
As Calcium Pantothenate	5.56 mg
Folic Acid	**400 mcg**
Folic Acid	0.49 mg
- Folic Acid	0.44 mg
- Absorbed Water	49 mcg
Vitamin C (Ascorbic Acid)	**90 mg**
As Ascorbic Acid 97%	100.21 mg
- Ascorbic Acid	97.20 mg
- Maize Starch	3.01 mg
Vitamin A (Retinol)	**700 mcg**
Vitamin A Concentrate Synthetic (Powder form)(analysed to 565 IU/mg)	5.21 mg
- Retinol Acetate	1.02 mg
- Sucrose	1.77 mg
- Gelatin	1.25 mg
- Modified Starch	0.83 mg
- Butylated Hydroxytoluene	0.07 mg
- Sodium Aluminosilicate	18 mcg
- Water	0.25 mg

Example 4

Effect of selenium on viability on storage:

The following mixtures have been stored in a dehumidified room at a temperature of 25 °C. Starting counts and counts of viable organisms after the indicated storage period were measured.

(a)

5mg LGG + 295mg Microcrystalline cellulose:

Start week 0: count $3,5x10^9$ Cfu/ tablet

End week 8: count $2,9x10^9$ Cfu/tablet

(b)

5mg LGG + 0.05mg Selenium + 295mg Microcrystalline cellulose:

Start week 0: count $4,0 x10^9$ Cfu/ tablet

End week 8: $4,6x10^9$ Cfu/tablet

It can be seen that the presence of selenium was beneficial to the stability of the micro-organisms, and indeed that the numbers of recoverable micro-organisms even increased on storage in the presence of selenium.

In each case the probiotic bacteria were *Lactobacillus rhamnosus GG* "Grade P" (ATCC 53103) as a concentrated, freeze-dried bacterial powder.

Claims

1. A probiotic tablet comprising a probiotic micro-organism and other nutritionally active ingredients, the tablet comprising at least two zones, a first of said zones comprising said probiotic micro-organism, and a second of said zones comprising at least one said other active ingredient kept separated from the probiotic micro-organism of said first zone, the water activity in said probiotic micro-organism containing first zone being no greater than 0.2 and the water content of said tablet being no less than 0.2% by weight.

2. A tablet as claimed in claim 1, wherein said first zone contains also selenium as a said at least one other active ingredient.

3. A tablet as claimed in claim 1 or claim 2, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of iron.

4. A tablet as claimed in any preceding claim, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of copper.

5. A tablet as claimed in any preceding claim, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of vitamin B6.

6. A tablet as claimed in any preceding claim, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of vitamin C.

7. A tablet as claimed in any preceding claim, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of zinc.

8. A tablet as claimed in any preceding claim, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of

manganese.

9. A tablet as claimed in any preceding claim, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of chromium.

10. A tablet as claimed in any preceding claim, wherein said first zone is free from amounts deleterious to the viability of the probiotic micro-organisms of pantothenic acid or its salts.

11. A tablet as claimed in any preceding claim, wherein said second zone contains iron as at least one said other active ingredient.

12. A tablet as claimed in any preceding claim, wherein said second zone contains vitamin B6 as at least one said other active ingredient.

13. A tablet as claimed in any preceding claim, wherein said second zone contains vitamin C as at least one said other active ingredient.

14. A tablet as claimed in any preceding claim, wherein said second zone contains copper as at least one said other active ingredient.

15. A tablet as claimed in any preceding claim, wherein said second zone contains manganese as at least one said other active ingredient.

16. A tablet as claimed in any preceding claim, wherein said second zone contains pantothenic acid or a salt thereof as at least one said other active ingredient.

17. A tablet as claimed in any preceding claim, wherein said second zone contains zinc as at least one said other active ingredient.

18. A tablet as claimed in any preceding claim, wherein said second zone contains chromium as at least one said other active ingredient.

19. A tablet as claimed in any preceding claim, wherein said second zone contains any two or more of iron, vitamin B6, vitamin C, pantothenic acid or a salt thereof, zinc, copper, chromium and manganese, each as at least one said other active ingredient.

20. A tablet as claimed in any preceding claim, wherein the probiotic micro-organism is mixed with a desiccant carrier material serving to reduce the water activity of the zone containing the probiotic micro-organism.

21. A tablet as claimed in any preceding claim, wherein the second zone contains a desiccant carrier material serving to reduce the water activity of the zone containing the probiotic micro-organism.

22. A tablet as claimed in claim 20 or claim 21, wherein said desiccant material comprises at least one of carboxymethylcellulose, colloidal silica, polyvinylpyrrolidone, starch, gelatine, hydroxypropylcellulose - low-substituted, microcrystalline cellulose, fumed silicon dioxide, sodium croscarmellose, crospovidone, povidone, magnesium aluminium silicate, methylcellulose, sodium alginate, sodium starch glyconate, gelatine, pregelatinized starch, or sorbitol.

23. A tablet as claimed in any preceding claim, having a multilayer structure comprising at least two layers, one of said layers constituting said first zone and another of said layers constituting said second zone.

24. A tablet as claimed in claim 23, wherein said layer constituting said first zone is free of encapsulated iron.

25. A tablet as claimed in claim 23 or claim 24, wherein said layer constituting said first zone is free of encapsulated zinc.

26. A tablet as claimed in any one of claims 23 to 25, wherein said layer constituting said first zone is free of encapsulated copper.

27. A tablet as claimed in any one of claims 23 to 26, wherein said layer constituting said first zone contains encapsulated vitamin B1.

28. A tablet as claimed in any one of claims 23 to 27, wherein said layer constituting said first zone contains encapsulated vitamin B6.

29. A tablet as claimed in any one of claims 23 to 28, wherein said layer constituting said first zone contains encapsulated zinc.

30. A tablet as claimed in any one of claims 23 to 29, wherein said layer constituting said first zone is contains encapsulated manganese.

31. A tablet as claimed in any one of claims 23 to 30, wherein said layer constituting said first zone contains encapsulated vitamin A, D, E, B12 or B2.

32. A tablet as claimed in any one of claims 1 to 22, having a multitude of granules constituting said first zone surrounded by a matrix, and wherein said matrix constitutes said second zone or wherein said matrix also contains a multitude of granules constituting said second zone.

33. A tablet as claimed in any preceding claim, wherein the water content of the tablet is at least 1% by weight.

34. A tablet as claimed in any preceding claim, wherein the water content of the tablet is at least 2% by weight.

35. A tablet as claimed in any preceding claim, wherein the water content of the tablet is at least 3% by weight.

36. A tablet as claimed in any preceding claim, wherein the water content of the tablet is no more than 4% by weight.

37. A tablet as claimed in any preceding claim, wherein the water content of the tablet is no more than 5% by weight.

38. A tablet as claimed in any preceding claim, wherein the water content of the tablet is no more than 6% by weight.

39. A tablet as claimed in any preceding claim, wherein the water content of the tablet is no more than 7% by weight.

40. A tablet as claimed in any preceding claim, wherein the water activity of said first zone is no greater than 0.15.

41. A tablet as claimed in claim 40, wherein the water activity of said first zone is no greater than 0.1.

42. A tablet as claimed in claim 41, wherein the water activity of said first zone is no greater than 0.05.

43. A tablet as claimed in claim 42, wherein the water activity of said first zone is no greater than 0.02.

44. A tablet as claimed in any preceding claim, wherein the water activity of the tablet is below 0.15.

45. A tablet as claimed in any preceding claim, wherein said first zone is separated from said second zone by a water excluding barrier material.

46. A tablet as claimed in any preceding claim, wherein the tablet is surrounded by a water excluding material.

47. A tablet as claimed in any preceding claim stored in a container containing a desiccant for absorbing water so as to reduce the water activity in the area surrounding said tablet, and/or containing an oxygen scavenging agent and/or

containing an inert gas atmosphere.

48. A tablet as claimed in any one of claims 45 to 47, wherein a said barrier material is a fat or wax based barrier material.

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49. A tablet as claimed in any preceding claim, wherein said first zone contains also one or more of iodine, magnesium, nicotinamide and folic acid as a said at least one other active ingredient.